The effect of Persian shallot (*Allium hirtifolium* Boiss.) extract on blood sugar and serum levels of some hormones in diabetic rats

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Abstract: Diabetes mellitus (DM) is caused by hyperglycemia, resulting from defective insulin secretion or function. It is widely believed that the antioxidant micronutrients obtained from plants afford significant protection against diseases like diabetes mellitus. Present study was aimed to examine the effects of Persian shallot (*Allium hirtifolium* Boiss) on FBS, HbA1c, insulin, Triiodothyronine (T3) and Thyroxine (T4) levels in type 1 diabetic rats. Thirty two male Wistar rats were divided into 4 groups of 8. The diabetic groups received 100 and 200 mg/kg Persian shallot extract, diabetic control and normal control received %0.9 saline for 30 days. At the end of treatments, fasting blood specimens were collected. The levels of FBS, HbA1c, insulin, T3 and T4 were measured. Our findings indicated that hydroalcoholic extract of Persian shallot significantly decreased serum levels of FBS and HbA1c in treated groups (in a dose dependent manner) ($P<0.05$). The serum levels of insulin and T3 slightly increased by Persian shallot but the T4 serum level was declined. These beneficial effects of Persian shallot extracts in diabetic rats could probably be due to the antioxidant capacity of its phenolic and diallyl disulfide content.

Keywords: *Allium hirtifolium* Boiss, Persian shallot, type 1 diabetes, insulin, hormone.

INTRODUCTION

Diabetes mellitus (DM), which is a chronic disease state caused by inherited or acquired deficiency in insulin secretion. Insulin deficiency in turn leading to decreased organ-specific response to insulin. DM therapy is very costly and approximately 5% of global population suffers from DM. Insulin deficiency causes elevation of blood glucose level, which this can impose damages to various organs (Leahy, 2005).

It is now well established that thyroid hormones regulate production of insulin and a relationship between serum thyroid hormone levels and risk of diabetes is also documented (Crunkhorn and Patti, 2008). Thyroid hormones regulate pancreas function including glucose homeostasis by interaction of thyroid hormone with its corresponding receptors (Handisurya *et al.,* 2008). Perros *et al*., reported that thyroid dysfunction is increased in type 1 diabetic adult females (1995). The main causes of hypothyroidism are most often Hashimoto's autoimmune thyroiditis disease. Since type 1 diabetes follows the pattern of autoimmunity (as a pathophysiological detonator), it is not unusual to find patients with concomitant diabetes and thyroid dysfunction (Pearce and Merriman, 2009).

In diabetes, tissue structure and function is damaged due to hyperglycemia. Hyperglycemia also causes non-enzymatic glycation changes to the structure and function of some soluble and insoluble proteins *in vivo* and *in vitro* (Haitoglou *et al.,* 1992). The elevated HbA1c level is a diagnostic biomarker for DM, HbA1c is one of the most valuable parameters for assessing glycemic control but the test is not particularly sensitive (Chandalia and Krishnaswamy, 2002). The benefits of medicinal plants with combined antiglycation and antioxidant properties in diabetic patients is reported (Nakagawa *et al*., 2002).

Since complete protocol for diabetes therapy without side effects is yet to be discovered and most of antidiabetic medications could have side effects (Cheng and Josse 2004). Investigations have been conducted to identify natural substances that show potent hypoglycemic activities with least side effects. Onions could apply as one of targets for anti-diabetic therapy via the antioxidant properties (Queiroz *et al.,* 2009). Garlic and shallot are widely used in parallel with main meal and also as a traditional medication in many countries. Garlic extracts is reported to be rich in flavonoids and sulfur-containing compounds (Kodera *et al*., 2002). Garlic and garlic constituents stated to have various biological activities, including anticarcinogenic, antithrombotic, antimicrobial, antioxidant, antidabetic (Azuma *et al.,* 2007).

Biochemical analysis of shallot extract demonstrated the presence of falvonols including quercetin, and sulfur compounds like diallyl disulfide in its content. Current knowledge regarding the properties and contents of

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shallot and its analogy with garlic suggests that some biological activities of shallot extracts are the same as garlic extracts. Study by Leelarungrayub *et al. (*2006) revealed that both shallot and garlic extract can significantly down regulate fasting blood glucose in insulin resistance rats. They also stated that aqueous shallot extract is a stronger hypoglycemic agent than the garlic extract, thus, it could be useful in insulin resistance state. Another study suggested that garlic supplementation significantly reduced serum glucose but increased serum insulin and liver glycogen. The hypoglycemic properties of garlic seem to be associated with the elevation of insulin level. The altered insulin response also activates glycogen synthetase and lead to conversion of blood glucose into glycogen. Therefore, it is clear that hormones are one of the most effective factors in the reduction of blood glucose (Mahmoodi *et al*., 2011).

Persian shallot (*Allium hirtifolium* Boiss) is a member of liliacea family with a specific taste. Persian shallot is yellow in color and oval in shape and contains a main bulb (rarely two bulbs) (Ebrahimi *et al*., 2008).

The Persian shallot (Mooseer) grows as a wild plant only in Zagros Mountains in central part of Iran and very little is known regarding its properties, specifically its related effect on hormones level in diabetes. Because hormones are one of the most effective factors in diabetes, this study was aimed to examine Persian shallot effects on controlling FBS, HbA1c, insulin, T3 and T4 in diabetic rats.

MATERIAL AND METHODS

Preparation of hydroalcoholic extract

Fresh Persian shallot (*Allium hirtifolium* Boiss) bulbs were obtained from Kangavar (Kermanshah, Iran). The genus and species of the bulbs were confirmed by the botanists (Department of botany, Valiasr University Rafsanjan-Iran). Then, 100 gr of fresh bulbs was well crushed and 400 ml distilled water/ethanol (25/75) was added. After 48 hours incubation, the solution was filtered using a filter paper through a Buchner funnel. The filtered resultant solutions obtained from this stage, concentrated by means of a vacuum distillation and decanted to dry powder, then, needed concentrations prepared (Momeni, 2000).

Animals and treatments

In this study 32 male Wistar rats (weighing between 180 to 230 g) were recruited. Twenty four rats received 45 mg/kg body weight of streptozotocin (STZ) (diabetic type-1 rats) and eight rats considered as normal group (Celik *et al*, 2009). After being matched according to body weight, the rats were divided into 4 groups of 8:

Group1: Diabetic rats received daily 200 mg/kg Persian shallot extract (2 ml) for 30 days.

Group 2: Diabetic rats received daily 100 mg/kg Persian shallot extract (2 ml) for 30 days.

Group 3: Diabetic rats received daily 0.9% saline (2 ml) for 30 days (diabetic control).

Group 4: Normal rats received daily 0.9% saline (2 ml) for 30 days (normal control).

The solutions (2ml) given to animals by a gavage syringe. Diabetes was confirmed by the measurement of serum glucose. Animals were then housed in cages and had freely access to water and food. Animal handling was performed with regard to Iranian animal ethics society and local university rules and under supervision of professor Mahmoodi who has the animal handling license. Following 30 days blood samples were collected and the levels of FBS, HbA1c, insulin, T3 and T4 was measured in all study groups.

Measurement of FBS and HbA1c

Plasma glucose level was estimated by BT-3000 instrument. Glycated hemoglobin was assessed using ion exchange micro-columns chromatography kit (Biosystem, Spain).

Measurement of insulin

The rats' serum insulin level was quantitatively measured using ELISA kits (Mercodia, Sweden).

Measurement of T3 and T4

Plasma T3 and T4 was estimated by radioimmunoassay (RIA) procedure (gammacounter-LKB).

STATISTICAL ANALYSIS

Differences in FBS, HbA1c, insulin, T3 and T4 level between control groups and case groups were analyzed by the one-way ANOVA. Data are expressed in terms of mean ± standard deviation (SD). P values less than 0.05 were considered statistically significant.

RESULTS

In current study we tried to explore the impact of Persian shallot on serum level of hormones, FBS and HbA1c in rats following 30 days Persian shallot extract consumption.

Body weight

There were significant differences in body weight among normal control with other three diabetic group, it observed slightly increase in diabetic groups after 30 days of Persian shallot feeding in dose dependent manner ($P<0.05$) (fig. 1).

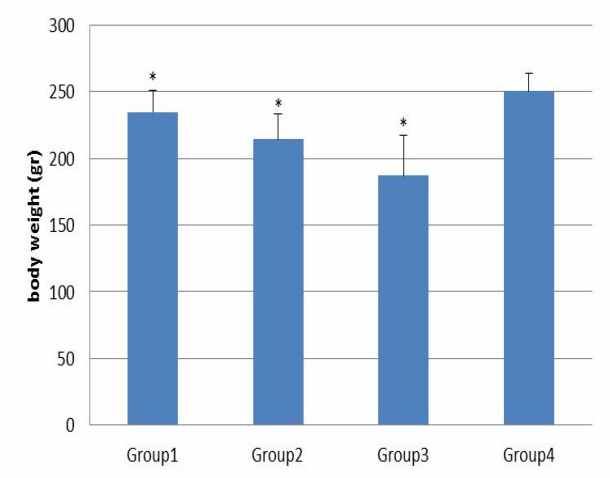

Fig. 1: The effect of different concentration of Persian shallot on body weight (gr) (Mean±SD).
Group1: diabetic rats received 200 mg/kg Persian shallot.
Group2: diabetic rats received 100 mg/kg Persian shallot.
Group3: diabetic rats received 0.9% saline.
Group4: normal rats received 0.9% saline.
*Significant differences with Group 4 (P<0.05)

FBS

The FBS concentrations of four groups of rats during experimental period are shown in fig. 2. There was a significant difference was observed between FBS level of all groups (P<0.05).

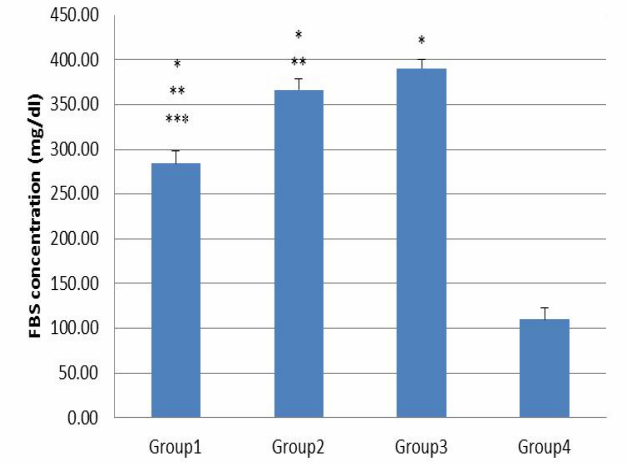

Fig. 2: The effect of different concentration of Persian shallot on FBS level (mg/dl). (Mean ± SD)
Group1: diabetic rats received 200 mg/kg Persian shallot.
Group2: diabetic rats received 100 mg/kg Persian shallot.
Group3: diabetic rats received 0.9% saline.
Group4: normal rats received 0.9% saline.
*Significant differences with Group 4 (P<0.05).
**Significant differences with Group 3 (P<0.05).
***Significant differences with Group 2 (P<0.05).

HbA1c

We also observed a significant difference in HbA1c level among normal and diabetic groups. Persian shallot also reduces HbA1c level significantly in diabetic group (dose dependent manner) (P<0.05).

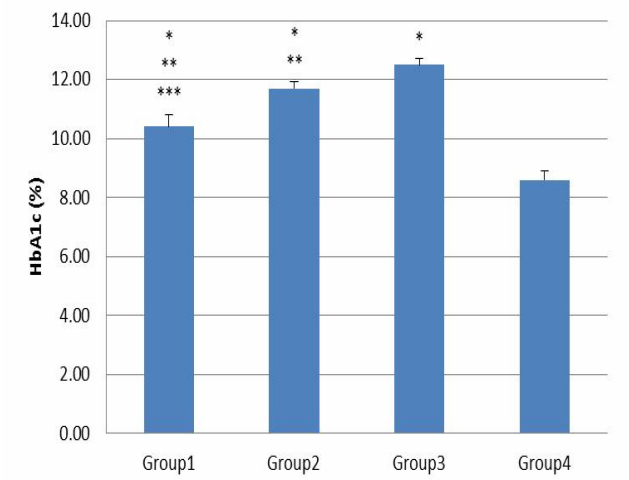

Fig. 3: The effect of different concentration of Persian shallot on HbA1c level (%) (Mean ± SD).
Group1: diabetic rats received 200 mg/kg Persian shallot.
Group2: diabetic rats received 100 mg/kg Persian shallot.
Group3: diabetic rats received 0.9% saline.
Group4: normal rats received 0.9% saline.
*Significant differences with Group 4 (P<0.05).
**Significant differences with Group 3(P<0.05).
***Significant differences with Group 2(P<0.05).

Fasting plasma insulin level

The Fasting plasma Insulin levels of all groups of rats during experimental period are displayed in fig. 4. Diabetic groups showed significantly lower insulin levels compare to normal control. However, there were not significant differences between Persian shallots treated groups (groups 1 and 2).

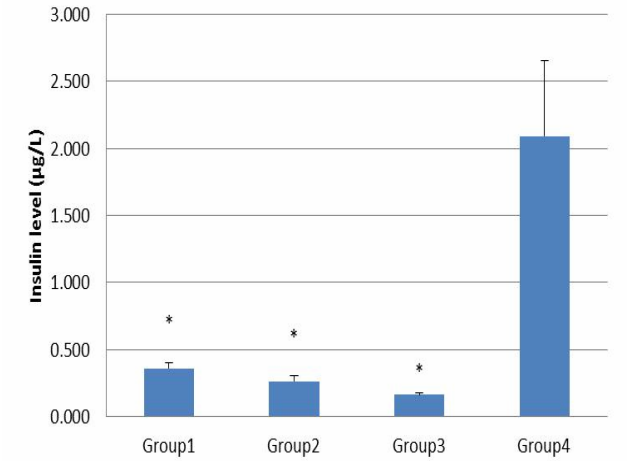

Fig. 4: The effect of different concentration of Persian shallot on Insulin level (IU/L) (Mean ± SD).
Group1: diabetic rats received 200 mg/kg Persian shallot.
Group2: diabetic rats received 100 mg/kg Persian shallot.
Group3: diabetic rats received 0.9% saline.
Group4: normal rats received 0.9% saline.
*Significant differences with Group 4(P<0.05).

T3 and T4 levels

The T3 and T4 levels of all groups are illustrated in fig. 5 and fig. 6, respectively. There were not any significant differences between 4 groups. However in diabetic group, Persian shallot gently increase T3 level and slightly decreased T4 level but these changes weren't significant.

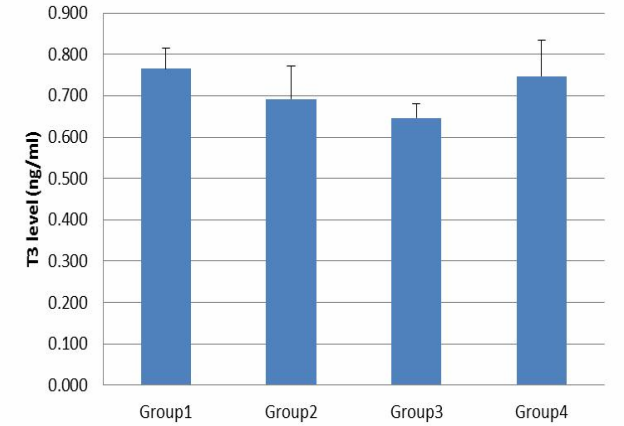

Fig. 5: The effect of different concentration of Persian shallot on T3 level (IU/L) (Mean ± SD).
Group1: diabetic rats received 200 mg/kg Persian shallot.
Group2: diabetic rats received 100 mg/kg Persian shallot.
Group3: diabetic rats received 0.9% saline.
Group4: normal rats received 0.9% saline.

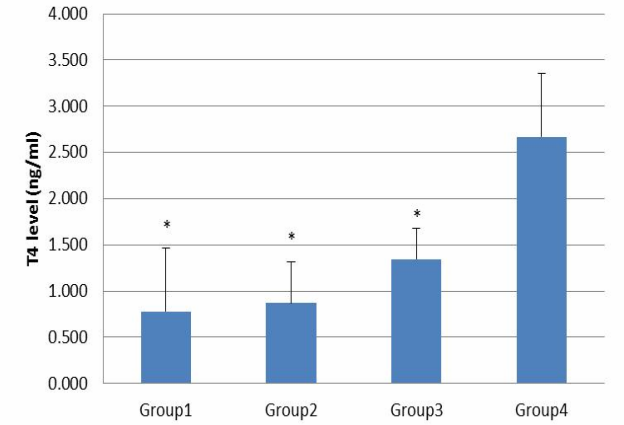

Fig. 6: The effect of different concentration of Persian shallot on T4 level (IU/L) (Mean ± SD).
Group1: diabetic rats received 200 mg/kg Persian shallot.
Group2: diabetic rats received 100 mg/kg Persian shallot.
Group3: diabetic rats received 0.9% saline.
Group4: normal rats received 0.9% saline.
*Significant differences with Group 4 ($P<0.05$).

DISCUSSION

In the current study induction of type 1 diabetes by STZ caused elevated FBS and HbA1c levels which reduced it insulin, T3 and T4 level in rats. STZ destroys pancreatic β-cells selectively and inhibits the synthesis and release of insulin, which in turn lead to onset of diabetes mellitus (Kavalali *et al*., 2002). There have been observed an inter link between insulin resistance (suppressed insulin-stimulated glucose uptake) to several disorders such as diabetes mellitus. Insulin is functionally important in adaptation to the ingestion of nutrients, dietary carbohydrates (Bessesen, 2001).

The impact of thyroid hormones on glucose metabolism has been known for a long time. Thyroid disorders are more prevalent in type 1 and 2 diabetes, due to the common autoimmune origin. On the other hand, a much higher frequency of sub-clinical hypothyroidism has been reported in metabolic syndrome patients (Brenta, 2010). Thyroid hormones exert their profound effects on the level of glucose homeostasis regulation. These effects are varying from modifications of circulating insulin levels and counter-regulatory hormones, intestinal absorption, hepatic production and peripheral tissues (fat and muscle) uptake of glucose. Thyroid hormones oppose the action of insulin and stimulate hepatic gluconeogenesis and glycogenolysis. They up-regulate some of genes including GlUT-4 and phosphoglycerate kinase, involving glucose transport and glycolysis, thus, acting synergistically with insulin (Clement *et al*., 2002).

Current finding indicate that treatment with Persian shallot extract can increases hormones T3 and insulin levels slightly while decreases the T4 level in diabetic rats. In consistent with our results Padiya *et al*. (2011) showed that garlic increased plasma insulin level in rats. But in our previous study we did not observe any significant changes in hormones level in garlic treated diabetic patients (Mahmoodi *et al.,* 2011). A previous study confirmed that shallot extracts act as antioxidant similar to (or slightly higher than) garlic extracts. The antioxidant properties of the garlic and shallot extracts are probably due to their phenolic and sulfur compounds (Leelarungrayub *et al.,* 2006, Hosseini *et al*., 2012). Previous studies reported that phenolic and diallyl disulfide compounds of shallot extract were higher than garlic (Leelarungrayub *et al*., 2004, Terrance *et al*., 1992). Administration of insulin to STZ-induced diabetic rats showed to increase serum thyroid hormone levels in compare to control animal (Chandalia and Krishnaswamy, 2002). So we suppose maybe shallot increased insulin level and then insulin raised the T4 level, but our finding showed adverse results about T3.

In addition to hormones, we measured FBS and HbA1c levels in all groups that shallot in dose dependent manner reduces significantly them in blood. Padiya *et al*., indicated that raw garlic homogenate elevated the insulin sensitivity while reduced metabolic complications and oxidative stress in diabetic rats, but not significantly affect the HbA1c level (2011). Hyperglycemia is crucial in pathogenesis of diabetes complications. Increased glycation and accumulation of tissue (Advance Glycated End Products) AGEPs cause changes enzyme activity via impairing its function and conformation. It also modifies

protein half-life, immunogenicity and structural cross-link of protein structures. Considerable interests raised in inhibitors of glycation for their therapeutic potential. Antiglycation compounds may act as blockers carbonyl groups on reducing sugars, amadori products, and 3-deoxy-glucosones to inhibit formation of AGEPs. Certain enzymes can deglycate amadori products and are referred as amadoriases. One can speculate Persian shallot may probably cleave AGEP cross-links, thus, leading diabetes complications. Antioxidants may protect against glycation-derived free radicals, whereas chelators remove transition metals, preventing autoxidation of glucose and Amadori products (Ahmad and Ahmed, 2006).

In an investigation, Ebrahimi *et al*. (2008) reported that Kangavar shallot contains more iron (Fe) and copper (Cu) compared to 17 different types of Persian shallots, obtained from different geographical regions of Iran (2008). Therefore, maybe some of the antioxidant properties of Persian shallot that observed in present study are related to these compounds. Polyphenolic compounds and flavonoids can protect the cells against emptying of reduced glutathione via elevated enzymes capacity. Furthermore, having antioxidant properties, these compounds are able to inactivate environment free radicals existing, hence, prevent their destructive effects (Baer-Dubowska *et al*., 1998).

CONCLUSION

In conclusion, the present study may indicate that treatment of rats with hydroalchoholic extract of shallot can reduced FBS, HbA1c significantly and changed slightly the levels of some hormones like Insulin, T3 and T4. Therefore, shallot extract seems, to be useful therapeutic reagent as an herbal medicine in prevention or diabetes therapy.

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